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PORTLAND HARBOR RI/FS
Round 3 Lamprey Ammocoete
Phase 2 Toxicity Testing Data Report

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May 7, 2008

Prepared for:
The Lower Willamette Group

Prepared by:
Windward Environmental LLC

WE-08-0003



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

REGION 10

1200 SIXTH AVENUE

SEATTLE, WA 98101

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May 15, 2008

Chip Humphrey
Eric Blischke
US Environmental Protection Agency, Region 10
811 SW Sixth Avenue, Third Floor
Portland, OR 97204

Re: Submittal of the Round 3 Lamprey Ammocoete Phase 2 Toxicity Testing Data Report

Dear Messrs. Humphrey and Blischke:

On behalf of the Lower Willamette Group, Windward Environmental LLC is pleased to submit the *Portland Harbor RI/FS Round 3 Lamprey Ammocoete Phase 2 Toxicity Testing Data Report*.

Sincerely,

A handwritten signature in black ink, appearing to read "Lisa Saban".

Lisa Saban
Partner

cc: Confederated Tribes and Bands of the Yakama Nation
Confederated Tribes of the Grand Ronde Community of Oregon
Confederated Tribes of Siletz Indians of Oregon
Confederated Tribes of the Umatilla Indian Reservation
Confederated Tribes of the Warm Springs Reservation of Oregon
Nez Perce Tribe
Oregon Department of Fish and Wildlife
United States Fish and Wildlife Service
Oregon Department of Environmental Quality



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Recommended for Inclusion in Administrative Record

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LIST OF ACRONYMS

ASTM	American Society for Testing and Materials
C	centigrade
CaCO ₃	calcium carbonate
CAS	Columbia Analytical Services, Inc.
EPA	US Environmental Protection Agency
FSP	field sampling plan
ft-c	foot candle
Integral	Integral Consulting, Inc.
LC50	concentration that is lethal to 50% of an exposed population
LWG	Lower Willamette Group
LWR	Lower Willamette River
NAS	Northwestern Aquatic Sciences
pps	pulses per second
QAPP	quality assurance project plan
RM	river mile
SD	standard deviation
TOC	total organic carbon
TRV	toxicity reference value
Windward	Windward Environmental LLC

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1.0 INTRODUCTION

Lamprey ammocoetes are the only detritivorous fish present in the Lower Willamette River (LWR). Four species of lamprey may exist in the LWR; of these, the Pacific lamprey (*Entosphenus tridentatus*, formerly known as *Lampetra tridentata*) was selected as the representative species for detritivorous fish (Integral et al. 2004). As outlined in the *Portland Harbor Remedial Investigation/Feasibility Study Programmatic Work Plan* (Integral et al. 2004), a tissue-residue approach was proposed to assess risks to lamprey ammocoetes. Pacific lamprey ammocoetes are the preferred tissue type, but if ammocoetes cannot be captured within the Portland Harbor area (River Mile [RM] 2.0 to RM 11), other fish tissue would be used as a surrogate. Because issues have been raised regarding whether the current ecological risk assessment approach to protect resident aquatic species will also be protective of lamprey ammocoetes, the US Environmental Protection Agency (EPA) and its partners have requested a sensitivity study of lamprey ammocoetes (*Lampetra* sp.). EPA specified the following two general objectives: 1) determine whether existing fish toxicity reference values (TRVs) are sufficiently protective of lamprey survival and growth as determined by laboratory testing with representative chemicals (the rationales for the selected chemicals are presented in the *Portland Harbor RI/FS Round 3 Lamprey Ammocoete Toxicity Testing Quality Assurance Project Plan* (Windward 2006)), and 2) identify the relative sensitivity of Pacific lamprey to adverse contaminant effects by comparing the data with published toxicity data for the most sensitive surrogate species (EPA 2006).

Phase 1 of the acute toxicity testing was conducted in the fall of 2006, and Phase 2 was conducted in the late summer and fall of 2007 and winter of 2008. This data report describes the objectives, methods, and procedures used during the Phase 2 toxicity testing with lamprey ammocoetes and the results of the testing.

1.1 OBJECTIVES OF TOXICITY TESTING

The specific objectives of the Phase 2 lamprey ammocoete collection and testing effort were to:

- Establish a proper exposure system for the flow-through tests, including the size of exposure chambers and rate of flow.
- Perform a range-finding toxicity test with naphthalene. Because of the high volatility of naphthalene, the range-finding test with naphthalene was not conducted during the Phase 1 toxicity testing effort but was delayed until Phase 2.
- Perform one acceptable (i.e., passing the acceptability criteria), definitive flow-through test to derive the concentration that is lethal to 50% of an exposed population

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(LC50) for each of the following six chemicals: copper, aniline, pentachlorophenol, naphthalene, diazinon, and lindane.

Phase 2 of the study was carried out because the first three objectives in Phase 1 were successfully accomplished (Windward 2007d). The concentrations of the five chemicals used in the flow-through definitive tests (i.e., copper, aniline, pentachlorophenol, diazinon, and lindane) were based on the range-finding tests conducted during Phase 1 (Windward 2007d). A proper exposure system was developed for the flow-through tests. A range-finding test was conducted with naphthalene prior to the definitive flow-through test.

1.2 REPORT ORGANIZATION

The remaining sections of this document describe the field sampling procedures, laboratory holding methods, and toxicity tests used for the lamprey ammocoetes. Section 2.0 presents the sampling procedures, and Section 3.0 describes laboratory methods. The toxicity test results are presented in Section 4.0, and species sensitivity distributions are presented in Section 5.0. Section 6.0 summarizes the conclusions; cited references are listed in Section 7.0. EPA-LWG communications are provided in Appendix A. Supporting information, including field collection logbooks and chain-of-custody forms are provided in Appendix B. The toxicity testing report from Northwestern Aquatic Sciences (NAS), including all raw laboratory data, is presented in Appendix C. Results of the chemical analysis of the holding sediment and water samples from each toxicity test are presented in Appendix D. The validation report for the toxicity testing is presented in Appendix E, and the validation report for the water chemistry is presented in Appendix F.

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2.0 FIELD METHODS

This section describes the methods used to collect lamprey ammocoetes in the field and presents the water quality parameters measured in the four coastal rivers.

2.1 AMMOCOETE COLLECTION

Lamprey ammocoetes were collected from four coastal rivers: Trask River, Nestucca River, Siletz River, and Alsea River. The ammocoetes were collected during two sampling events, August 27-30 and October 15-18, 2007. During each sampling event, a total number of 1,000 ammocoetes were targeted for collection from the rivers as follows: Trask and Nestucca Rivers, 250 organisms each; Siletz River, 300 organisms; and Alsea River, 200 organisms (Figure 2-1). The sampling locations were selected based on the Phase 1 sampling effort and the reconnaissance survey (Windward 2007c, 2007d). The sampling depth, velocity, and water and sediment temperature were measured before the sampling effort was initiated at each location. The substrate was mostly medium to fine sands mixed with silt and some leaf litter. The sampling water depths ranged from approximately 2 cm to 95 cm.



Figure 2-1. Lamprey (*Lampetra* sp.) Ammocoete Sampling Locations

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0 1 2 3 4 5 10 15 Miles
0 2.5 5 10 15 20 Kilometers



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The sampling methods were the same as those used during the Phase 1 field effort. A Smith-Root, Inc., LR-24 dual-train backpack electroshocker was used. An initial setting of three pulses per second (pps) and 25% duty cycle was selected to withdraw the ammocoetes from the sediment, and a second setting of 30 pps and 25% duty cycle was applied to stun the ammocoetes. The stunned ammocoetes were then captured with a small fishing net. The length of the ammocoetes was estimated, and only ammocoetes in the appropriate size range were retained. Captured ammocoetes were placed in a pre-cleaned cooler partially filled with site water and approximately 2 in. of site sediment. Fifty ammocoetes were placed in each cooler. Frozen gel packs were affixed to the inside lids of the coolers to keep the water temperature cool during holding and transport to the laboratory. At the end of the sampling day, the coolers were filled with additional site water before being transported to NAS in Newport, Oregon. Sampling information and water quality parameters measured upon arrival at the laboratory for the August and October sampling events are presented in Tables 2-1 and 2-2, respectively.

Table 2-1. Lamprey Ammocoete Information from the August Sampling Event

Parameter	Trask River	Nestucca River	Siletz River	Alsea River
Sampling date	8/27/07	8/28/07	8/29/07	8/30/07
Sampling location	lower Trask boat ramp	Three Rivers boat ramp	Morgan boat ramp	downstream of Hellion Rapids
Northing	45°25.818N	45°14.079N	44°47.974N	44°23.584N
Easting	123°49.131W	123°52.456W	123°54.432W	123°49.840W
Ammocoete field count	251	251	309	200
Ammocoete laboratory count	260	258	312	202
Ammocoete captured, handled, and released count	88	302	234	177
Substrate	medium to fine silty sand	medium to fine silty sand with leaf debris	sandy gravel with some silt	silty fine sand with leaf debris
Field water temperature (°C)	15 – 17	15 – 20	19 – 22	19 – 21
Field sediment temperature (°C)	15	15	17	17
Water sampling depth (cm)	3 – 43	5 – 50	5 – 50	2 – 50
Velocity (m/sec)	0.0 – 0.3	0.0 – 0.3	0.0 – 0.5	0.0 – 0.3
Laboratory temperature (°C) ^a	18.1	17.1	18.1	16.9
Hardness (mg/L as CaCO ₃) ^a	30	28	22	31

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Table 2-1. Lamprey Ammocoete Information from the August Sampling Event

Parameter	Trask River	Nestucca River	Siletz River	Alsea River
Alkalinity (mg/L as CaCO ₃) ^a	25	10	16	25
pH (unitless) ^a	6.7	6.6	6.1	6.6
Dissolved oxygen (mg/L) ^a	8.6	9.8	7.2	9.0
Conductivity (µmhos/cm) ^a	82	68	60	60

^a Measured in the coolers upon delivery to the laboratory.

CaCO₃ – calcium carbonate

Table 2-2. Lamprey Ammocoete Information from the October Sampling Event

Parameter	Trask River	Nestucca River	Siletz River	Alsea River
Sampling date	10/15/07	10/16/07	10/17/07	10/18/07
Sampling location	Lower Trask boat ramp	Three Rivers boat ramp and Wayside boat ramp	"Miller Place"	downstream of Hellion Rapids, Campbell Park, and Salmonberry boat ramp
Northing	45°25.818 N	45°14.086N and 45°14.081N	44°47.983N	44°23.588N, 44°21.948N, and 44°20.568N
Easting	123°49.131W	123°52.450W and 123°52.406W	123°56.906W	123°23.588W, 123°41.385W, and 123°41.206W
Ammocoete field count	251	270	319	67
Ammocoete laboratory count	251	265	323	66
Ammocoete captured, handled, and released count	109	93	164	48
Substrate	sandy silt	silty sand with gravel and leaf debris and sand with silt and gravel	silty sand	silty fine sand and silty sand
Field water temperature (°C)	13	11	11	10 – 12
Field sediment temperature (°C)	12	10 – 11	11	10
Water sampling depth (cm)	53 – 80	5 – 45	5 – 41	5 – 95
Velocity (m/sec)	0.0 – 0.1	0.0 – 0.3	0.0	0.0

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Table 2-2. Lamprey Ammocoete Information from the October Sampling Event

Parameter	Trask River	Nestucca River	Siletz River	Alsea River
Laboratory temperature (°C) ^a	13.0	12.4	13.3	13.1
Hardness (mg/L as CaCO ₃) ^a	30	18	60	20
Alkalinity (mg/L as CaCO ₃) ^a	30	14	55	14
pH (unitless) ^a	6.7	6.2	8.3	6.8
Dissolved oxygen (mg/L) ^a	10.6	8.9	10.1	10.2
Conductivity (µmhos/cm) ^a	97	65	135	57

^a Measured in the coolers upon delivery to the laboratory.CaCO₃ – calcium carbonate

During the first sampling event, EPA requested that all captured ammocoetes be measured for length to provide more accurate information on the size distribution in each river. Figures 2-2 through 2-5 present the size distributions from each of the four rivers based on all ammocoetes captured (both retained and released). EPA was kept informed during the field effort on the size of the captured ammocoetes. When it became apparent that a sufficient number of ammocoetes could not be collected in the size range specified in the field sampling plan (FSP) addendum (2.5 to 4.0 cm) (Windward 2007b), EPA approved an increase in the size range to 2.5 to 6.0 cm (Shephard 2007a). EPA later limited the sizes to be used in the toxicity tests to 4.0 to 6.0 cm (Shephard 2007b). During the second sampling event, only ammocoetes close to this size range were captured, and only ammocoetes with a length of 4 to 6 cm were retained.

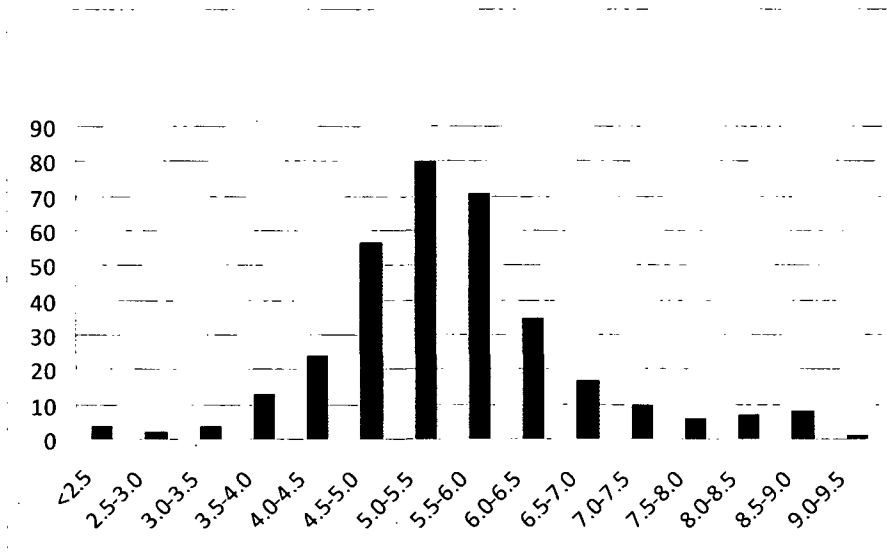


Figure 2-2. Size Distribution of Lamprey Ammocoetes in Trask River Based on All Ammocoetes Captured in August

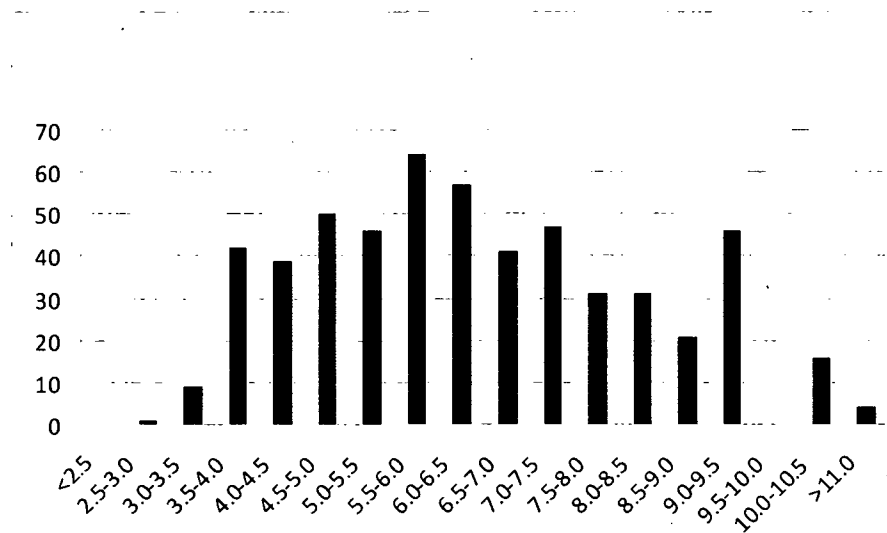


Figure 2-3. Size Distribution of Lamprey Ammocoetes in Nestucca River Based on All Ammocoetes Captured in August

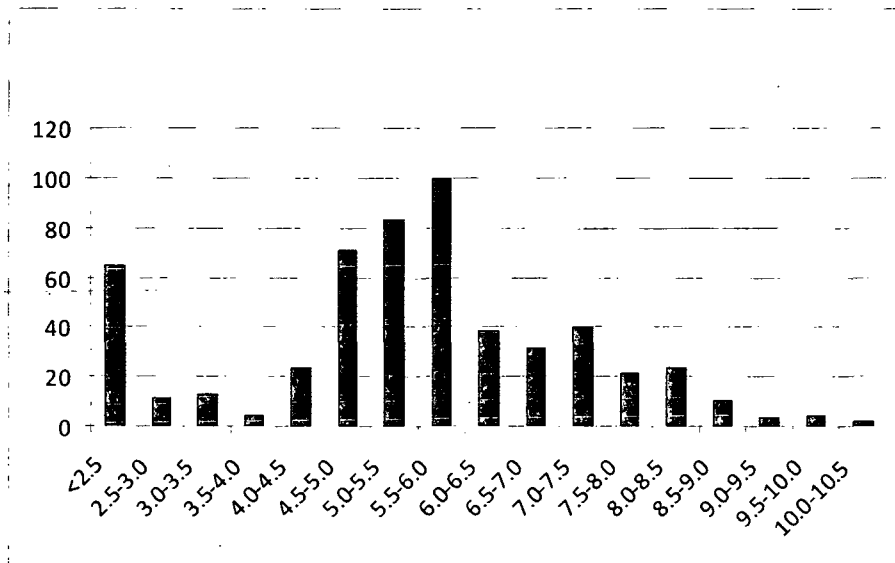


Figure 2-4. Size Distribution of Lamprey Ammocoetes in Siletz River Based on All Ammocoetes Captured in August

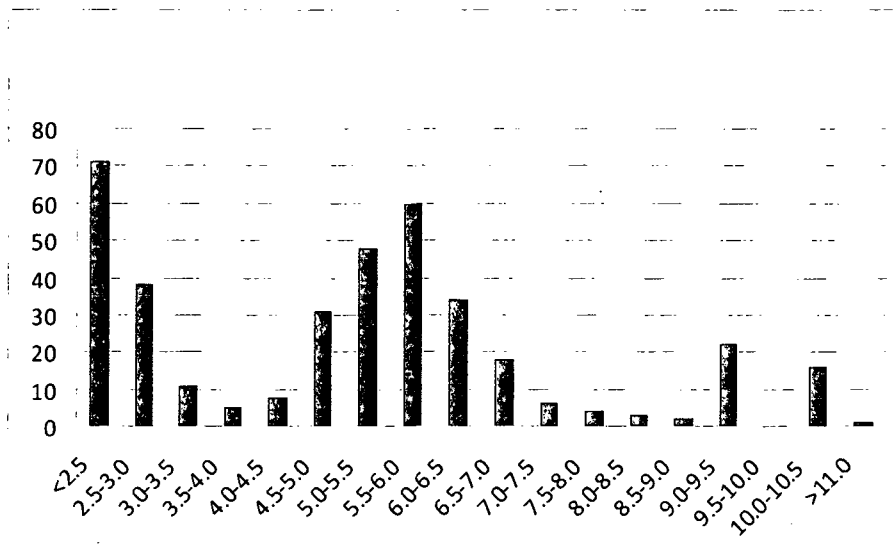


Figure 2-5. Size Distribution of Lamprey Ammocoetes in Alsea River Based on All Ammocoetes Captured in August

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2.2 DEVIATIONS FROM THE FSP

The collection of lamprey ammocoetes in the field was performed with one deviation from the FSP addendum (Windward 2007b). During the first sampling effort in the four coastal rivers, it became apparent that sufficient ammocoetes could not be collected in the 2.5-to-4.0-cm size range; and the range during this collection effort was increased to 2.5 to 6.0 cm upon approval from EPA (2007). During the second sampling effort, ammocoetes that were within a size range of 4.0 to 6.0 cm were retained based on a direction from EPA (Shephard 2007a).

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3.0 LABORATORY METHODS

This section describes the methods used to hold the lamprey ammocoetes in the laboratory, the toxicity and temperature testing methods, the analytical methods of the confirmatory water samples, and deviations from the FSP and quality assurance project plan (QAPP).

3.1 LAMPREY AMMOCOETE HOLDING

The first sampling effort was conducted in August when the water temperatures in the rivers were close to the testing temperature of 17 °C. The water temperatures measured in the coolers upon arrival at the laboratory ranged between 16.9 and 17.1 °C, and the ammocoetes were transferred directly into the 10-gal. holding tanks, with a distribution of approximately 50 organisms per tank. The tanks contained approximately 8 cm of sediment and 26 L of water that was aerated. The holding sediment had been analyzed for pesticides prior to the collection of ammocoetes. Only a trace amount (0.16J µg/kg) of endosulfan sulfate was detected (Appendix D). The water was supplied using a flow-through system at a rate of approximately 35 mL/minute (approximately two volume changes per day). In addition, two-thirds of the water volume in each tank were replaced daily. The tanks were held at a temperature of 16.7 ± 0.4 °C and ambient laboratory lightning (i.e., 50 to 73 foot-candles [ft-c]) at a daily photoperiod of 16 hours of light and 8 hours of darkness.

The second sampling effort was conducted in October when the water temperatures in the rivers ranged from 10 to 12 °C. The drop in temperature was expected; and in preparation for the arrival of the new batch of ammocoetes, the laboratory had grouped all remaining ammocoetes from the 20 holding tanks into a few tanks in a separate environmental chamber, which was kept at the testing temperature (17 ± 1 °C). The water temperature in the 20 holding tanks and in the initial environmental chamber was then lowered to 12 °C; all other conditions were similar to the holding conditions for the first batch of ammocoetes. The water temperature measured in the coolers upon arrival at the laboratory ranged between 12.4 and 13.3 °C, and the ammocoetes were transferred directly into the 10-gal. holding tanks, with a distribution of approximately 50 organisms per tank. After the laboratory had received all the ammocoetes collected in the four rivers, the temperature in the 20 holding tanks was increased 1 °C per day until the temperature reached the testing temperature of 17 ± 1 °C.

The ammocoetes were acclimated in the laboratory for 2 to 3 weeks after collection before testing was initiated. Normal behavior for lamprey ammocoetes is to immediately burrow in sediment and remain there. If any individuals are observed swimming around and not burrowing, it is an indication that the ammocoetes are under some sort of stress. All ammocoetes not burrowing were considered to be potentially damaged and were immediately removed. Lamprey ammocoetes were not fed during holding on the recommendation of William Swink, MS, a research fishery biologist with the US

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Geological Survey at the Hammond Bay Biological Station.¹ Table 3-1 summarizes the water quality parameters in the tanks during ammocoete holding. The results of the chemical analysis of the holding sediment are provided in Appendix D, and additional details on the ammocoete holding conditions are presented in Appendix C.

Table 3-1. Water Quality Parameters During Ammocoete Holding

Parameter	Unit	Mean \pm SD
Temperature	°C	16.7 \pm 0.4
Dissolved oxygen	mg/L	9.5 \pm 0.5
pH	unitless	7.8 \pm 0.4
Conductivity	μ mhos/cm	160 \pm 24
Hardness	mg/L as CaCO ₃	55 \pm 12
Alkalinity	mg/L as CaCO ₃	39 \pm 9

C – centigrade

CaCO₃ – calcium carbonate

SD – standard deviation

3.2 LAMPREY AMMOCOETE TESTING

Phase 2 toxicity testing consisted of definitive tests with the six chemicals (i.e., copper, aniline, lindane, naphthalene, pentachlorophenol, and diazinon). In addition, a static renewal test with naphthalene was performed prior to the definitive test to determine the chemical concentrations for the definitive test. The definitive tests were 96-hour flow-through tests (water-only tests). The tests were conducted with a control and five concentrations in a 0.5 dilution series. The tests included four replicates that contained 10 ammocoetes for each concentration and the control. The loading rate ranged from 0.33 to 0.40 g of organism/L. These loading rates are well below the EPA (2002) loading rate limit of 6.1 g/L of tank capacity and the ASTM (1996) recommendation of < 1 g/L of solution passing through the exposure tanks in 24 hours. The range-finding test with naphthalene included one replicate that contained five ammocoetes for each of four test concentrations and the control. The loading rate in the range-finding test was 0.45 g of organism/L. All tests were conducted in soft water (26 to 30 mg/L as CaCO₃), at a temperature of 17° \pm 1.0 °C, and in the dark, except for approximately 2 to 3 hours each day during which water quality measurements and mortality observations were made and the light intensity at the aquaria level ranged between 13.2 and 15.7 ft-c. The ammocoetes were not fed during the tests. The numbers of live and dead ammocoetes were counted daily and at test termination in the control and the test concentrations. A subsample of 10 ammocoetes were weighed and measured at the beginning of the test. The average length ranged between 4.8 and 5.0 mm, and the average weight ranged between 0.19 and 0.23 g. Additional information on the testing conditions, including all

¹ Mr. Swink has over 15 years of experience working with sea lamprey, involving the culture of larval lampreys; effects of density on growth of larvae; and survival, growth, and feeding of newly metamorphosed lampreys.

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raw laboratory data, is presented in Appendix C; and the validation report for the toxicity testing is presented in Appendix E.

The nominal concentrations of the six chemicals used in the definitive tests were based on their respective range-finding test. The nominal concentrations prepared for each chemical by the toxicity testing laboratory were confirmed through chemical analysis. Water samples were collected daily from the control and each test concentration. Additional information on chemical purity and the preparation of stock solutions is presented in Appendix C. The confirmatory water chemistry data provided by Columbia Analytical Services, Inc. (CAS), of Kelso, Washington, including the ammonia, total organic carbon (TOC), and total suspended solids (TSS) data, are presented in Appendix D.

Because there is no standard protocol for acute toxicity testing with lamprey ammocoetes, the test procedures were based on methods for measuring acute toxicity with other fish species as described in EPA (2002) and American Society for Testing and Materials (ASTM) (1996) guidance. The test conditions and test acceptability criterion are summarized in Table 3-2.

Table 3-2. Summary of Test Conditions for the 96-Hour Definitive Flow-Through Toxicity Tests with Lamprey Ammocoetes

Parameter	Condition or Regimen
Test type	flow-through and static renewal (naphthalene)
Test duration	96 hours
Temperature	17° ± 1° C
Light quality	ambient laboratory
Illuminance	none
Photoperiod	24 hours of darkness (except during daily measurements and observations)
Test chamber size	7.6-L glass aquaria covered with Plexiglas® plates
Solution volume	5.8 L per aquarium
Renewal of test solution	continuously or in the naphthalene range-finding test, at 48 hours
Test organisms	lamprey ammocoetes
Number of test treatments	5 test treatments for the definitive test; 4 treatments for the range-finding test
Number of replicates per treatment	4 replicates for the definitive tests; 1 replicate for the range-finding test
Organisms per replicate	10 organisms per replicate in the definitive test; 5 organisms per replicate in the range-finding test
Number of organisms per exposure concentration	40 organisms per exposure concentration in the definitive tests; 5 organisms per exposure concentration in the range-finding test
Test chamber cleaning	none
Feeding	none
Aeration	gentle aeration not to exceed 100 bubbles per minute
Dilution water	de-chlorinated municipal tap water with a hardness of 45 ± 9 mg/L spiked

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Table 3-2. Summary of Test Conditions for the 96-Hour Definitive Flow-Through Toxicity Tests with Lamprey Ammocoetes

Parameter	Condition or Regimen
	with the selected chemicals
Test concentrations	5 test concentrations and a control or for the naphthalene range-finding test, 4 concentrations and a control
Endpoint	survival
Test acceptability criterion	≥ 90% survival in the control

3.3 ANALYTICAL METHODS FOR TOXICITY TEST WATER

The nominal test solution concentrations used in each 96-hour definitive test and the range-finding test for naphthalene were confirmed by CAS. Test solution samples were collected daily during testing and shipped to CAS. At the initiation and termination of each test, all solution samples were also analyzed for particulate matter (i.e., TSS), TOC, and ammonia. CAS also completed these water quality tests. Samples were analyzed according to the methods presented in Table 3-3.

Table 3-3. Laboratory Methods for the Analysis of Toxicity Test Solution Samples

Chemical	Laboratory Method	
	Sample Preparation	Quantitative Analysis
Copper ^a	EPA 3005/CLP	EPA 200.8
Lindane	EPA 3535	EPA 8081A
Diazinon	EPA 3520C	EPA 8141A
Aniline	EPA 3520C	EPA 8270C
Pentachlorophenol	EPA 8151M	EPA 8151M
Naphthalene	EPA 5035	EPA 8260B
Total suspended solids	na	EPA 160.2
Total organic carbon	na	EPA 415.1
Ammonia	na	EPA 350.1

^a For copper, both filtered and unfiltered samples were analyzed.

CLP – Contract Laboratory Program

EPA – US Environmental Protection Agency

na – not applicable

Prior to the beginning of the Phase 2 lamprey ammocoete toxicity testing, a sample of the same lot of commercial sand used for holding in the Phase 1 toxicity testing was analyzed by CAS to verify that it was free of herbicides and pesticides. The sediment sample was analyzed according to the methods presented in Table 3-4.

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Table 3-4. Laboratory Methods for the Analysis of Holding Sediment

Chemical	Laboratory Method	
	Sample Preparation	Quantitative Analysis
Organochlorine pesticides	EPA 3541	EPA 8081A
Chlorinated herbicides	EPA 8151A	EPA 8151A

EPA – US Environmental Protection Agency\

Data validation was completed by EcoChem in Seattle, Washington. Data quality was acceptable and met the objectives of the lamprey toxicity study. EcoChem's data validation report is provided in Appendix F.

3.4 DEVIATIONS FROM THE FSP AND QAPP

Two deviations from the FSP and QAPP occurred in all six definitive tests. The protocol specified that the tests should be conducted in constant darkness, except during monitoring activities, at which time the light intensity should be 50 to 75 ft-c. Because of the possibility that the specified light intensity could induce fright behavior in the ammocoetes, the light at the aquaria was reduced to an intensity level that ranged between 13.2 and 15.7 ft-c during monitoring activities. The protocol specifies that at the end of the test, all surviving control ammocoetes should be weighed and measured for length. Through a laboratory error, 10 ammocoetes (a subsample) were weighed and measured at the beginning of the test instead. This is a minor deviation and is without consequence for the interpretation of the study results. At the end of the lindane test, the diluter flows were not inspected; however, lindane concentrations and water quality data all confirmed the satisfactory performance of the diluter.

Chemical analyses of all samples were completed as described in the Portland Harbor RI/FS Round 2 Quality Assurance Project Plan Addendum 7: Round 3 Chemical Analysis of Lamprey Ammocoete Toxicity Test Water (Integral 2006) and Portland Harbor RI/FS Round 3 Lamprey Toxicity Testing Quality Assurance Project Plan Addendum: Phase 2 Lamprey Ammocoete Collection and Testing (Windward 2007a) with the following exceptions. Additional water volume was not collected at a frequency of 5% of the samples for chemistry laboratory quality control. When insufficient sample volume was received for duplicate or matrix spike analysis, the laboratory substituted the analysis of duplicate laboratory control samples. The highest concentration of diazinon sampled at day 3 and measured by CAS was three orders of magnitude lower than the nominal concentration. This deviation had little effect on the test because all ammocoetes in the highest concentration were dead by day 2. The measured concentration was omitted from the LC50 calculation.

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4.0 TESTING RESULTS

The test results for the definitive flow-through tests with the five chemicals tested in Phase 1 are presented in Section 4.1, and the test results from the toxicity tests conducted with naphthalene are presented in Section 4.2.

4.1 DEFINITIVE FLOW-THROUGH TESTS

This section presents the test results and water quality parameters documented during the definitive flow-through tests with the five chemicals tested in Phase 1. Temperature, dissolved oxygen, and pH were measured daily in all concentrations. Conductivity, hardness, and alkalinity were measured daily in the control and highest concentration.

4.1.1 Copper

The 96-hour definitive flow-through test with copper was initiated on September 14, 2007, and terminated on September 18, 2007. The daily survival rates documented throughout the test and the nominal and mean measured chemical concentrations are presented in Table 4-1. The test met the acceptability criterion of $\geq 90\%$ survival in the control. An LC50 value of 46 $\mu\text{g/L}$ was calculated based on the survival data at test termination. The concentrations of copper measured throughout the test are presented in Table 4-2; water quality parameters are summarized in Table 4-3.

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Table 4-1. Daily Survival of Ammocoetes and Chemical Concentrations of Dissolved and Total Copper

Concentration (µg/L)			Number of Surviving Ammocoetes					Percent Survival
Nominal	Dissolved Measured Mean ± SD	Total Measured Mean ± SD	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours	
0 (control)	0.82 ± 0.63	0.75 ± 0.57	40	40	40	40	40	100
12.5	13 ± 0.62	14 ± 0.20	40	40	40	40	40	100
25	23 ± 1.1	24 ± 0.79	40	40	40	40	40	100
50	43 ± 3.2	46 ± 1.9	40	40	40	40	23	57.5
100	88 ± 9.0	95 ± 4.6	40	40	40	27	0	0
200	164 ± 36	188 ± 16	40	40	40	4	0	0

SD – standard deviation

Table 4-2. Dissolved and Total Copper Concentrations Throughout the Definitive Flow-Through Test

Nominal Concentration (µg/L)	Measured Concentration (µg/L)									
	0 Hour		24 Hours		48 Hours		72 Hours		96 Hours	
	Dissolved	Total	Dissolved	Total	Dissolved	Total	Dissolved	Total	Dissolved	Total
0 (control)	1.8	1.64	0.53 ^a	0.51 ^a	0.30	0.36	1.08	0.99	0.40 ^a	0.26
12.5	12.9	13.9	12.3	13.5	12.5	13.5	13.9	13.9	12.9	13.7
25	21.6	23.3	21.9	23.7	22.8	24.3	24.3	24.9	23.5	25.2
50	38.4 ^a	43.8 ^a	41.6	44.5	41.7	45.3	45.6	47.8	46.0	47.8
100	82.2	89.6	77.4	93.9	87.1	92.8	96.0 ^a	98.4 ^a	98.6	101
200	120	162	133	185	175	189	190	201	201	203

^a Average including a duplicate.

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Table 4-3. Water Quality Parameters During the Definitive Flow-Through Test with Copper

Parameter	Unit	Mean \pm SD
Temperature	°C	16.9 \pm 0.2
Dissolved oxygen	mg/L	8.6 \pm 0.5
pH	unitless	7.8 \pm 0.4
Conductivity	μ mhos/cm	102 \pm 7
Hardness	mg/L as CaCO ₃	27 \pm 1
Alkalinity	mg/L as CaCO ₃	18 \pm 4

C – centigrade

CaCO₃ – calcium carbonate

SD – standard deviation

4.1.2 Aniline

The 96-hour definitive flow-through test with aniline was initiated on October 1, 2007, and terminated on October 5, 2007. The daily survival rate documented throughout the test and the nominal and mean measured chemical concentrations are presented in Table 4-4. The test met the acceptability criterion of $\geq 90\%$ survival in the control. An LC50 value of 430 mg/L was calculated based on the survival data at test termination. The concentrations of aniline measured throughout the test are presented in Table 4-5; water quality parameters are summarized in Table 4-6.

Table 4-4. Daily Survival of Ammocoetes and Chemical Concentrations of Aniline

Concentration (mg/L)		Number of Surviving Ammocoetes					Percent Survival
Nominal	Measured Mean \pm SD	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours	
0 (control)	0.5 \pm 0.7	40	40	40	40	40	100
75	113 \pm 16	40	40	40	40	40	100
150	176 \pm 23	40	40	40	40	40	100
300	318 \pm 25	40	40	40	37	30	75
600	634 \pm 85	40	40	36	13	7	17.5
1,200	1,300 \pm 187	40	0	0	0	0	0

SD – standard deviation

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Table 4-5. Aniline Concentrations Throughout the Definitive Flow-Through Test

Nominal Concentration (mg/L)	Measured Concentration (µg/L)				
	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours
0 (control)	1.8	0.4	0.2	0.3	0.1
75	100	95	130	110	130
150	200	200	160	170	150
300	320	360	310	300	300
600	730	710	580	620	530
1,200	1,500	1,500	1,200	1,200	1,100

Table 4-6. Water Quality Parameters During the Definitive Flow-Through Test with Aniline

Parameter	Unit	Mean ± SD
Temperature	°C	16.9 ± 0.4
Dissolved oxygen	mg/L	9.2 ± 0.2
pH	unitless	6.4 ± 0.2
Conductivity	µmhos/cm	98 ± 2
Hardness	mg/L as CaCO ₃	26 ± 1
Alkalinity	mg/L as CaCO ₃	21 ± 1

C – centigrade

CaCO₃ – calcium carbonate

SD – standard deviation

4.1.3 Lindane

The 96-hour definitive flow-through test with lindane was initiated on October 18, 2007, and terminated on October 22, 2007. The daily survival rate documented throughout the test and the nominal and mean measured chemical concentrations are presented in Table 4-7. The test met the acceptability criterion of $\geq 90\%$ survival in the control. Because of low mortality, only an estimated LC50 of ≥ 2.68 mg/L could be derived. The concentrations of lindane measured throughout the test are presented in Table 4-8. The nominal concentrations were based on an initial estimate of the lindane saturation level at test temperature (approximately 8 mg/L). Numerous unvalidated direct measurements of lindane concentration in the Veith column eluates during the test, and in the mixing tank following a full 24-hour cessation of solution delivery to the diluter at the end of the test, suggested that the lindane water saturation level under test conditions was approximately 5 mg/L. The measured concentrations were lower than the suggested saturation level (5 mg/L) because actual saturation of the mixing tank contents with lindane could not be fully achieved based on the limited recirculation rate through the Veith columns and the constant addition of make-up water to replace solution delivered to the diluter. Water quality parameters are summarized in Table 4-9.

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Table 4-7. Daily Survival of Ammocoetes and Chemical Concentrations of Lindane

Concentration (mg/L)		Number of Surviving Ammocoetes					Percent Survival
Nominal	Measured Mean \pm SD	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours	
0 (control)	0.05 \pm 0.05	40	40	40	40	40	100
0.5	0.21 \pm 0.05	40	40	40	40	40	100
1.0	0.28 \pm 0.12	40	40	40	40	40	100
2.0	0.56 \pm 0.08	40	40	40	40	40	100
4.0	1.15 \pm 0.340	40	40	40	40	40	100
8.0	2.68 \pm 0.733	40	40	40	35	35	87.5

SD – standard deviation

Table 4-8. Lindane Concentrations Throughout the Definitive Flow-Through Test

Nominal Concentration (μ g/L)	Measured Concentration (μ g/L)				
	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours
0 (control)	0.009	0.001	0.009	0.001	0.001
0.5	0.15	0.17	0.26	0.25	0.22
1.0	0.33	0.34	0.36	0.07	0.31
2.0	0.67	0.44	0.56	0.57	0.58
4.0	0.76	1.00	1.60	1.00	1.40
8.0	2.50	2.70	3.60	3.00	1.60

Table 4-9. Water Quality Parameters During Definitive Flow-Through Test with Lindane

Parameter	Unit	Mean \pm SD
Temperature	$^{\circ}$ C	17.3 \pm 0.2
Dissolved oxygen	mg/L	8.9 \pm 0.4
pH	unitless	6.9 \pm 0.2
Conductivity	μ mhos/cm	96 \pm 4
Hardness	mg/L as CaCO ₃	26 \pm 1
Alkalinity	mg/L as CaCO ₃	15 \pm 1

CaCO₃ – calcium carbonate

SD – standard deviation

4.1.4 Pentachlorophenol

The 96-hour definitive flow-through test with pentachlorophenol was initiated on December 16, 2007, and terminated on December 20, 2007. The daily survival rate documented throughout the test and the nominal and mean measured chemical concentrations are presented in Table 4-10. The test met the acceptability criterion of

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≥ 90% survival in the control. An LC50 value of 31 µg/L was calculated based on the survival data at test termination. The concentrations of pentachlorophenol measured throughout the test are presented in Table 4-11; water quality parameters are summarized in Table 4-12.

Table 4-10. Daily Survival of Ammocoetes and Chemical Concentrations of Pentachlorophenol

Concentration (µg/L)		Number of Surviving Ammocoetes					Percent Survival
Nominal	Measured Mean ± SD	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours	
0 (control)	0 ± 0.0	40	40	40	40	40	100
12.5	17 ± 4	40	40	40	40	40	100
25	30 ± 6	40	40	40	40	20	50
50	56 ± 12	40	40	35	11	1	2.5
100	110 ± 14	40	33	0	0	0	0
200	230 ± 25	40	0	0	0	0	0

SD – standard deviation

Table 4-11. Pentachlorophenol Concentrations Throughout the Definitive Flow-Through Test

Nominal Concentration (µg/L)	Measured Concentration (µg/L)				
	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours
0 (control)	0	0	0	0	0
12.5	22	20	15	14	16
25	38	29	28	23	30
50	73	50	50	45	55
100	130	115	110	95	100
200	250	260	230	210	200

Table 4-12. Water Quality Parameters During the Definitive Flow-Through Test with Pentachlorophenol

Parameter	Unit	Mean ± SD
Temperature	°C	17.0 ± 0.3
Dissolved oxygen	mg/L	9.9 ± 0.1
pH	unitless	6.6 ± 0.2
Conductivity	µmhos/cm	125 ± 2
Hardness	mg/L as CaCO ₃	30 ± 2
Alkalinity	mg/L as CaCO ₃	15 ± 1

C – centigrade

CaCO₃ – calcium carbonate

SD – standard deviation

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4.1.5 Diazinon

The 96-hour definitive flow-through test with diazinon was initiated on January 16, 2008, and terminated on January 20, 2008. The daily survival rate documented throughout the test and the nominal and mean measured chemical concentrations are presented in Table 4-13. The test met the acceptability criterion of $\geq 90\%$ survival in the control. An LC50 value of 8.90 mg/L was calculated based on the survival data at test termination. The concentrations of diazinon measured throughout the test are presented in Table 4-14. Measured test concentrations were between 63 and 72% of nominal concentrations. The difference between nominal and measured concentrations was likely caused by the adsorption of diazinon to the walls and tubes of the mixing tank and the distribution system and incomplete solution of diazinon in the mixing tank. Water quality parameters are summarized in Table 4-15.

Table 4-13. Daily Survival of Ammocoetes and Chemical Concentrations of Diazinon

Concentration (mg/L)		Number of Surviving Ammocoetes					Percent Survival
Nominal	Measured Mean \pm SD	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours	
0 (control)	0.0 \pm 0.0	40	40	40	40	40	100
2.5	1.7 \pm 0.1	40	40	40	40	40	100
5	3.2 \pm 0.2	40	40	40	40	40	100
10	6.3 \pm 0.5	40	40	40	40	39	97.5
20	13 \pm 2.8	40	40	40	20	1	2.5
40	29 \pm 7.7	40	40	0	0	0	0

SD – standard deviation

Table 4-14. Diazinon Concentrations Throughout the Definitive Flow-Through Test

Nominal Concentration (mg/L)	Measured Concentration (mg/L)				
	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours
0 (control)	0.0	0.0	0.0	0.0	0.0
2.5	1.5	1.7	1.7	1.8	1.7
5	3.0	3.5	3.0	3.4	3.3
10	5.5	6.4	6.4	6.6	6.7
20	8.5	12	12	14	16
40	23	27	25	0.04 ^a	40

^a This concentration was omitted from the LC50 calculation.

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Table 4-15. Water Quality Parameters During Definitive Flow-Through Test with Diazinon

Parameter	Unit	Mean \pm SD
Temperature	°C	16.9 \pm 0.41
Dissolved oxygen	mg/L	9.8 \pm 0.1
pH	unitless	7.3 \pm 0.1
Conductivity	μ mhos/cm	114 \pm 6
Hardness	mg/L as CaCO ₃	30 \pm 0
Alkalinity	mg/L as CaCO ₃	10 \pm 1

C – centigrade

CaCO₃ – calcium carbonate

SD – standard deviation

4.2 TOXICITY TESTS WITH NAPHTHALENE

This section presents the test results and water quality parameters documented during the toxicity tests with naphthalene. Section 4.2.1 provides the results from the range-finding test, and Section 4.2.2 provides the results from the definitive flow-through test. Temperature, dissolved oxygen, and pH were measured daily in all concentrations. Conductivity, hardness, and alkalinity were measured daily in the control and highest concentration.

4.2.1 Range-Finding Test

The 96-hour static renewal range-finding test with naphthalene was initiated on October 30, 2007, and terminated on November 3, 2007. The daily survival rate documented throughout the test and the nominal and mean measured chemical concentrations are presented in Table 4-16. The test met the acceptability criterion of $\geq 90\%$ survival in the control. The concentrations of naphthalene measured throughout the test are presented in Table 4-17. Because naphthalene is very volatile, the nominal concentrations could not be achieved, as indicated by the measured concentrations. Water quality parameters are summarized in Table 4-18.

Table 4-16. Daily Survival of Ammocoetes and Chemical Concentrations of Naphthalene in the Range-Finding Test

Concentration (mg/L)		Number of Surviving Ammocoetes					Percent Survival
Nominal	Measured Mean \pm SD	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours	
0 (control)	0.00 \pm 0.00	5	5	5	5	5	100
0.03	0.03 \pm 0.03	5	5	5	5	5	100
0.3	0.14 \pm 0.06	5	5	5	5	5	100

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Table 4-16. Daily Survival of Ammocoetes and Chemical Concentrations of Naphthalene in the Range-Finding Test

Concentration (mg/L)		Number of Surviving Ammocoetes					Percent Survival
Nominal	Measured Mean \pm SD	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours	
3.0	1.4 \pm 0.64	5	5	5	5	5	100
30	15 \pm 4.8	5	5	5	4	2	40

SD – standard deviation

Table 4-17. Chemical Concentrations of Naphthalene Throughout the Range-Finding Test

Nominal Concentration (mg/L)	Measured Concentration (μ g/L)					
	0 Hour	24 Hours	48 Hours – Old Solution	48 Hours – New Solution	72 Hours	96 Hours
0 (control)	0.00	0.00	0.00	0.00	0.00	0.00
0.03	0.10	0.02	0.02	0.02	0.02	0.02
0.3	0.09	0.17	0.07	0.20	0.20	0.09
3.0	1.1	2.1	0.63	2.0	1.9	0.89
30	13	17	8.9	22	18	11

Table 4-18. Water Quality Parameters During Range-Finding Test with Naphthalene

Parameter	Unit	Mean \pm SD
Temperature	°C	16.5 \pm 0.3
Dissolved oxygen	mg/L	8.1 \pm 0.3
pH	unitless	7.2 \pm 0.1
Conductivity	μ mhos/cm	93 \pm 8
Hardness	mg/L as CaCO ₃	22 \pm 1
Alkalinity	mg/L as CaCO ₃	12 \pm 3

C – centigrade

CaCO₃ – calcium carbonate

SD – standard deviation

4.2.2 Definitive Flow-Through Test

The 96-hour definitive flow-through test with naphthalene was initiated on November 14, 2007, and terminated on November 18, 2007. The daily survival rate documented throughout the test and the nominal and mean measured chemical concentrations are presented in Table 4-19. The test met the acceptability criterion of $\geq 90\%$ survival in the control. Because of low mortality, only an estimated LC50 of ≥ 10.18 mg/L could be derived. The concentrations of naphthalene measured throughout the test are presented in Table 4-20. Because naphthalene is very volatile, the nominal concentrations could not

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be achieved, as indicated by the measured concentrations. Water quality parameters are summarized in Table 4-21.

Table 4-19. Daily Survival of Ammocoetes and Chemical Concentrations of Naphthalene

Concentration (mg/L)		Number of Surviving Ammocoetes					Percent Survival
Nominal	Measured Mean \pm SD	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours	
0 (control)	0.04 \pm 0.00	40	40	40	40	40	100
1.25	0.70 \pm 0.10	40	40	40	40	40	100
2.5	1.2 \pm 0.18	40	40	40	40	40	100
5.0	2.6 \pm 0.28	40	40	40	40	40	100
10	5.3 \pm 0.63	40	40	40	40	40	100
20	10 \pm 1.9	40	40	37	34	20	50

SD – standard deviation

Table 4-20. Naphthalene Concentrations Throughout the Definitive Flow-Through Test

Nominal Concentration (mg/L)	Measured Concentration (mg/L)				
	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours
0 (control)	0.04	0.04	0.04	0.04	0.04
1.25	0.63	0.81	0.81	0.69	0.58
2.5	1.1	1.4	1.4	1.3	1.0
5.0	2.4	2.9	2.9	2.7	2.3
10	5.0	6.2	5.5	5.4	4.5
20	7.4	12	12	10	9.5

Table 4-21. Water Quality Parameters During the Definitive Flow-Through Test with Naphthalene

Parameter	Unit	Mean \pm SD
Temperature	°C	17.2 \pm 0.4
Dissolved oxygen	mg/L	9.4 \pm 0.2
pH	unitless	7.3 \pm 0.2
Conductivity	μ mhos/cm	109 \pm 7
Hardness	mg/L as CaCO ₃	28 \pm 20
Alkalinity	mg/L as CaCO ₃	16 \pm 1

C – centigrade

CaCO₃ – calcium carbonate

SD – standard deviation

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5.0 CONCLUSIONS

The lamprey toxicity study met the specific objectives presented in Section 1.1: establish a proper exposure system for the six flow-through definitive tests, perform a range-finding test with naphthalene, and perform one acceptable (i.e., passing the acceptability criterion) definitive flow-through test for the six chemicals. LC50s were derived for copper, aniline, pentachlorophenol, and diazinon. An LC50 could not be derived for either naphthalene or lindane because the mortality in the highest concentration was $\leq 50\%$.

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